Immunogenicity and Reactogenicity of 1 versus 2 Doses of Trivalent Inactivated Influenza Vaccine in Vaccine-Naive 5–8-Year-Old Children

Kathleen M. Neuzil, Lisa A. Jackson, Jennifer Nelson, Alexander Klimov, Nancy Cox, Carolyn B. Bridges, John Dunn, Frank DeStefano, and David Shay

1Program for Appropriate Technology in Health, 2Department of Medicine, School of Medicine, and 3Department of Biostatistics, School of Public Health, University of Washington, and 4Center for Health Studies and 5Department of Pediatrics, Group Health Cooperative, Seattle, Washington; 6Influenza Branch, 7National Immunization Program, and 8Immunization Safety Office, Centers for Disease Control and Prevention, Atlanta, Georgia

(See the editorial commentary by Edwards and Griffin, on pages 1027–9.)

Background. Two doses of trivalent inactivated influenza vaccine (TIV) are recommended for children <9 years old receiving vaccine for the first time, but compliance is suboptimal. This study assessed the need for a second dose of TIV in this age group.

Methods. In this prospective, open-label study, 232 influenza vaccine–naive 5–8-year-olds enrolled in a health maintenance organization received 2 doses of TIV in fall 2004. Serum for antibody titer measurement was obtained at 3 time points (n = 222). Parents completed diaries for 5 days.

Results. Both doses of vaccine were well tolerated. The strongest predictor of a protective antibody response (≥1:40) after 1 dose of TIV was baseline seropositive status. In multivariate analysis adjusting for age, sex, and baseline serostatus, the proportion of children with protective antibody responses was significantly higher after 2 doses than after 1 dose of TIV for each antigen (P < .001, for A/H1N1; P = .01, for A/H3N2; P < .001, for B). Age and sex were not independently predictive of a protective antibody response. Over one-third of children had antibody responses <1:40 for the type B vaccine component, even after 2 doses.

Conclusions. The present study supports the need for 2 doses of TIV in 5–8-year-olds receiving TIV for the first time. Efforts to increase compliance with the 2-dose recommendation are warranted.
Influenza Vaccine in Children

Figure 1. Proportion of children with seropositive titers (≥1:10) for each influenza vaccine antigen at baseline, by age: 5 years (n = 55), 6 years (n = 50), 7 years (n = 58), and 8 years (n = 59).

or because they live with 1 or more persons at increased risk of influenza complications, including children <5 years old, adults 65 years and older, and persons of any age with certain chronic medical conditions [14]. As the recommendation to vaccinate all children 6 months through 4 years of age is implemented, the number of school-aged children who receive influenza vaccine each year is also expected to increase substantially.

Currently, the Centers for Disease Control and Prevention’s (CDC) Advisory Committee on Immunization Practices (ACIP) and the American Academy of Pediatrics (AAP) recommend that children younger than 9 years who have never been vaccinated with influenza vaccine receive 2 doses of influenza vaccine the first year they are vaccinated [14, 15]. This recommendation is based on the premise that a significant proportion of such children are immunologically naive to influenza virus infection and require 2 doses of inactivated influenza vaccine for an adequate immune response [10, 16, 17]. Although the scientific support for 2 doses of vaccine in infants and young children has been established, the need for 2 doses of vaccine for adequate immunogenicity in older children is less certain, and few contemporaneous studies have evaluated this age group specifically. From a practical standpoint, delivering 2 doses of vaccine before the influenza season is challenging [16]. Thus, we assessed the additional benefit derived from a second dose of trivalent inactivated influenza vaccine (TIV) in vaccine-naïve 5–8-year-old children.

SUBJECTS, MATERIALS, AND METHODS

Study design. The study was a prospective, open-label comparison of the immunogenicity and reactogenicity of 1 versus 2 doses of 2004–2005 TIV in 5–8-year-old children conducted at Group Health Cooperative (GHC), Seattle. The primary goal of this study was to compare the antibody response after 1 dose of influenza vaccine with that after 2 doses of influenza vaccine.

A secondary aim was to describe and compare the safety profile after 1 and 2 doses of vaccine. The study protocol was approved by the GHC and CDC institutional review boards, and informed consent was obtained from a parent or guardian of all study participants.

Study subjects. Children who were enrolled in GHC since birth, had no record of influenza vaccination in the GHC immunization registry, and would be 5–8 years old at the time of the first vaccination were invited to participate. Children were enrolled if parents gave informed consent, planned to be available for all study visits, and had telephone access. Subjects with acute febrile illnesses were eligible for enrollment, but vaccination was deferred for 24 h after the last oral temperature >38.0°C. Children were excluded from enrollment if there was a history of active cancer; an immunocompromising illness or current receipt of immunosuppressive agents; previous receipt of influenza vaccine of any kind; or allergy to eggs, egg products, or any other component of influenza vaccine.

Procedures. The recommended strains for the 2004–2005 influenza vaccine were A/New Caledonia/20/99 (H1N1)–like, A/Fujian/411/2003 (H3N2)–like, and B/Shanghai/361/2002-like strains. A single lot of licensed 2004–2005 TIV (A/New Caledonia/20/99 [H1N1], A/Wyoming/3/2003 [H3N2], an A/Fujian/411/2003-like vaccine strain), and B/Jiangsu/10/2003 [a B/Shanghai/361/2002-like vaccine strain]) provided by Sanofi Pasteur was used throughout the study. Subjects received 2 doses of 0.5 mL of TIV, 4 weeks apart. Vaccine was injected into the deltoid muscle using a 25-gauge, 1-inch needle. Blood samples were obtained from children at 3 time points—before receipt of dose 1, 4 weeks after receipt of dose 1 and before dose 2, and 4 weeks after dose 2. Hemagglutination inhibition (HAI) antibody titers to the A/H3N2, A/H1N1, and B antigens included in the vaccine were measured at each time point. HAI antibody titers were also determined for a B antigen (B/Hong Kong/307/1999,anto A/Panama/2007/99 [H1N1], and A/Beijing/219/2002 [H3N2] strains).
suspected of having a protective antibody response, relative risk regression was used. Specifically, we modeled the probability of a protective response as an exponential function of risk factors and performed nonlinear least squares estimation to obtain asymptotically unbiased estimates of the relative risk of a protective response. To account for misclassification of the variance and the correlation between observations for the same participant after dose 1 and dose 2, we computed model-robust (Huber-White) SEs and performed estimation using generalized estimating equation (GEE) methodology. We did not use a logistic regression function because the prevalence of a protective response is not rare, and the odds ratios obtained from logistic regression models will overestimate the relative risk except when the relative risk is zero [19]. Similarly, multivariate linear regression was used to model the GMTs as a function of dose, age, sex, and baseline serostatus. Estimation was performed using GEE, and model-robust SEs were computed to account for correlation between measures on the same participant after dose 1 and dose 2.

Safety analysis. The proportions of subjects with solicited adverse events of any severity were determined for each age and baseline antibody titer group after each dose. Analyses were primarily descriptive; however, we compared the proportions of children with any fever, local reaction, or moderate/severe pain after dose 1 with the proportions of children with such reactions after dose 2, by use of McNemar’s test. Additionally, after each dose, the proportions of children with local reactions or moderate/severe pain were compared between age groups and between baseline antibody titer groups by use of \( \chi^2 \) tests, respectively. GMTs and proportions were compared after dose 1 versus dose 2 by use of a paired \( t \) test and McNemar’s test, respectively. To assess the multivariate effects that dose, age (5–6 vs. 7–8 years), sex, and baseline serostatus have on protective antibody response, relative risk regression was used.

Laboratory methods. Serum samples were stored frozen at \(-20^\circ\text{C}\) or lower until analyzed at the CDC. HAI antibody titers were determined in duplicate, running all paired specimens in the same test. Before testing, serum samples were treated with receptor-destroying enzyme (RDE; Denke Seiken). To inactivate the RDE, samples were heated to \(56^\circ\text{C}\) for 30 min. After the treatment, the serum samples were diluted to 1:10 and were then subjected to 2-fold serial dilutions. Twenty-five microliters of the diluted serum samples was incubated with an equal volume of each of 4 strains: A/New Caledonia/20/99 (H1N1); A/Wyoming/3/2003 (H3N2); B/Jilin/20/2003, a B/Shanghai/361-like (B/Yamagata/16/88-lineage) reference virus; and B/Hong Kong/1434/2001, a B/Victoria/2/87-lineage reference virus. Each virus was diluted to contain 8 HA units, and 50 \( \mu\text{L} \) of a 0.5% suspension of turkey red blood cells was then added to the mixture [18].

Immunogenicity analysis. The proportions of subjects with a protective antibody response (\(\geq 1:40\)) and the geometric mean titers (GMTs) of HAI antibodies were determined for each antigen after each vaccine dose by age and baseline antibody titer (\(<1:10, \text{seronegative}; \geq 1:10, \text{seropositive}\)). After each dose, GMTs and the proportions of children with a protective antibody response were compared between age groups and between baseline antibody titer groups by use of \( \chi^2 \) tests and \( \chi^2 \) tests, respectively. GMTs and proportions were compared after dose 1 versus dose 2 by use of a paired \( t \) test and McNemar’s test, respectively. To assess the multivariate effects that dose, age (5–6 vs. 7–8 years), sex, and baseline serostatus have on protective antibody response, relative risk regression was used. Specifically, we modeled the probability of a protective response as an exponential function of risk factors and performed nonlinear least squares estimation to obtain asymptotically unbiased estimates of the relative risk of a protective response. To account for misclassification of the variance and the correlation between observations for the same participant after dose 1 and dose 2, we computed model-robust (Huber-White) SEs and performed estimation using generalized estimating equation (GEE) methodology. We did not use a logistic regression function because the prevalence of a protective response is not rare, and the odds ratios obtained from logistic regression models will overestimate the relative risk except when the relative risk is zero [19]. Similarly, multivariate linear regression was used to model the GMTs as a function of dose, age, sex, and baseline serostatus. Estimation was performed using GEE, and model-robust SEs were computed to account for correlation between measures on the same participant after dose 1 and dose 2.

Safety analysis. The proportions of subjects with solicited adverse events of any severity were determined for each age and baseline antibody titer group after each dose. Analyses were primarily descriptive; however, we compared the proportions of children with any fever, local reaction, or moderate/severe pain after dose 1 with the proportions of children with such reactions after dose 2, by use of McNemar’s test. Additionally, after each dose, the proportions of children with local reactions or moderate/severe pain were compared between age groups and between seronegative and seropositive children, by use of \( \chi^2 \) tests. Finally, relative risk regression was used to assess the joint effects that sex, age, and baseline titer have on the probability of any pain or of moderate/severe pain. Separate regression models were developed for such reactions after each dose.

RESULTS

A total of 280 children were enrolled in the study; 17 dropped out after consent but before the first vaccine dose, 31 dropped
Figure 2. Proportion of children with protective antibody responses (≥1:40) to each antigen included in the influenza vaccine, by dose, serostatus at baseline, and age.

Out after dose 1 but before dose 2, and 9 dropped out after receiving both vaccine doses but before the third blood draw. One child received both doses of vaccine but did not have a first blood sample obtained. Thus, 222 children who received 2 doses of TIV and had 3 blood samples available were included in our final immunogenicity analyses. Study diaries were returned after each dose for all 232 children who received 2 doses of TIV, and all 232 children were included in our final safety analyses.

The mean age of the 222 children at the time of first vaccine dose was 6.5 years, and 57% were male. Before the receipt of influenza vaccine, the proportions of children who were ser-
ropositive (HAI titer $\geq 1:10$) for A/H1N1, A/H3N2, and B vaccine antigens were 54%, 91%, and 30%, respectively. The proportion of children seropositive for vaccine antigens at baseline increased with increasing age (figure 1).

The proportion of children with protective antibody responses ($\geq 1:40$) after the first and second doses of vaccine are shown in table 1. The effect that dose had on protective antibody response differed significantly by baseline serostatus. Among children who were seronegative at baseline, the antibody responses were low after 1 TIV dose, whereas, among children who were seropositive at baseline, 95%, 100%, and 100% achieved protective antibody responses to A/H1N1, A/H3N2, and type B vaccine antigens, respectively, after only 1 dose.

In multivariate analysis adjusting for age, sex, number of doses, and baseline serostatus, among all children combined, the proportion with protective antibody responses was significantly higher after 2 doses than after 1 dose of TIV for each antigen ($P < .001$, for A/H1N1; $P = .01$, for A/H3N2; $P < .001$, for B). Likewise, a positive baseline titer to a specific antigen was significantly associated with the development of a protective antibody response to that antigen ($P < .001$, for all 3 antigens) (figure 2). After adjusting for baseline titer, neither age nor sex was independently predictive of a protective antibody response.

When antibody response was assessed by GMTs, results were similar, with high GMTs after 1 dose of vaccine in the seropositive group and low GMTs in the seronegative group (table 2). Although a second dose of vaccine significantly increased GMTs for the strains included in the vaccine in seronegative children, there were no significant increases in HAI GMTs after the second dose in seropositive children. Moreover, children who were seropositive at baseline had significantly higher HAI GMTs after receiving 1 dose of vaccine than did children who were seronegative at baseline after receiving 2 doses of vaccine. For both seronegative and seropositive children, HAI GMTs were significantly lower for the B vaccine component, than for the A components. Antibody responses to the B/Hong Kong strain not contained in this year’s vaccine were uniformly poor (tables 1 and 2).

Influenza vaccine was well tolerated in all age groups and after both doses (table 3). There were no significant differences in the proportion of children who had redness, swelling, fever, or itching after dose 1 than after dose 2. The proportions of children with any pain and with moderate/severe pain were significantly higher after dose 2 ($P = .002$ and $P < .001$, McNemar’s test, respectively). These differences were driven by differences in the older age groups (5–6-year-olds: $P = .08$, for any pain; $P = .012$, for moderate/severe pain; 7–8-year-olds: $P = .017$, for any pain; $P < .001$, for moderate/severe pain). No child reported pain beyond day 3 after vaccination. After ad-

<table>
<thead>
<tr>
<th>Antigen, baseline serostatus</th>
<th>Geometric mean HAI antibody titer (95% CI)</th>
<th>Before dose 1</th>
<th>After dose 1</th>
<th>After dose 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/H1N1$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>222</td>
<td>14 (12–17)</td>
<td>149 (111–200)</td>
<td>276 (229–334)</td>
</tr>
<tr>
<td>Seronegative</td>
<td>103</td>
<td>5$^b$</td>
<td>21 (16–29)</td>
<td>87 (70–107)</td>
</tr>
<tr>
<td>Seropositive</td>
<td>119</td>
<td>35 (31–40)</td>
<td>803 (658–980)</td>
<td>753 (656–865)</td>
</tr>
<tr>
<td>A/H3N2$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>222</td>
<td>52 (45–59)</td>
<td>360 (301–432)</td>
<td>421 (372–476)</td>
</tr>
<tr>
<td>Seronegative</td>
<td>19</td>
<td>5$^b$</td>
<td>9 (4–19)</td>
<td>48 (29–81)</td>
</tr>
<tr>
<td>Seropositive</td>
<td>203</td>
<td>64 (58–71)</td>
<td>509 (465–558)</td>
<td>516 (475–561)</td>
</tr>
<tr>
<td>B/Jilin$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>222</td>
<td>8 (7–9)</td>
<td>25 (20–32)</td>
<td>48 (40–57)</td>
</tr>
<tr>
<td>Seronegative</td>
<td>155</td>
<td>5$^b$</td>
<td>10 (8–11)</td>
<td>26 (22–31)</td>
</tr>
<tr>
<td>Seropositive</td>
<td>67</td>
<td>23 (19–27)</td>
<td>237 (207–272)</td>
<td>201 (175–231)</td>
</tr>
<tr>
<td>B/Hong Kong$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>222</td>
<td>5 (5–6)</td>
<td>7 (6–7)</td>
<td>7 (6–7)</td>
</tr>
<tr>
<td>Seronegative</td>
<td>210</td>
<td>5$^b$</td>
<td>6 (5–6)</td>
<td>6 (5–7)</td>
</tr>
<tr>
<td>Seropositive</td>
<td>12</td>
<td>17 (11–26)</td>
<td>40 (25–63)</td>
<td>40 (26–61)</td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval.

$^a$ Antigen contained in 2004–2005 trivalent inactivated influenza vaccine.

$^b$ Assigned value, no variation.

$^c$ Nonvaccine antigen.
## DISCUSSION

Recommendations for the appropriate use of TIV in children are based principally on the results of large multicenter trials of monovalent and bivalent influenza vaccines conducted in the 1970s [10, 14, 20, 21]. Influenza vaccines have changed considerably since that time. Current US-licensed influenza vaccines are trivalent, contain 15 μg of each antigen, and are exclusively subvirion products. This prospective clinical study is, to our knowledge, the first to compare the immunogenicity and reactogenicity of 1 versus 2 doses of modern TIV in this age group and affirm the recommendations of the ACIP and AAP for 2 doses of TIV in children younger than 9 years of age receiving influenza vaccine for the first time.

The strongest predictor of antibody response after a single dose of TIV was influenza-antigen serostatus at baseline. The primary benefit derived from the second dose of vaccine was to increase the proportion of seronegative children who achieved a protective antibody response. In this study, among initially seronegative children, an additional 50%, 51%, and 31% developed protective responses to the A/H1N1, A/H3N2, and B antigens, respectively, after the second TIV dose. In the subset of children who were seropositive at baseline, the second dose of vaccine did not increase the antibody levels over those achieved after the first dose (table 2). Unfortunately, there is no practical way to identify the serostatus of children when they present for vaccination. Age is correlated with baseline antibody titer, but this correlation is not strong enough to be a clinically useful surrogate. For example, even among the 8-year-olds in this cohort, 35% were seronegative for A/H1N1 and 50% were seronegative for B at baseline. In contrast, among all ages, only 9% of children were seronegative for A/H3N2 at baseline. These data reflect that A/H3N2 has been the predominant circulating influenza strain in the United States for the past 5–8 years, which represents the lifetime of these children [14]. Our study sample from a West Coast health maintenance organization may not be generalizable to all children in the United States, because influenza attack rates and circulation of types and subtypes of influenza viruses can vary substantially from community to community in a given season and from season to season. In addition, children in low-
income settings may have greater exposure to respiratory viruses at a younger age, which might increase the proportion of such children who are seropositive for influenza antigens by a given age [22].

Although we did not study vaccine efficacy, the correlation between level of virus-specific antibody titers and degree of protection is well established for influenza [23–26]. It would be expected that the improved antibody responses observed in children after 1 or 2 doses of TIV will lead to improved protection, and a recent study of the effectiveness of 1 versus 2 doses of TIV in children 6 months to 8 years old supports our results [27]. In addition, studies of influenza outbreaks among children demonstrate that, at any given antibody level, protection after natural infection may be superior to protection after immunization with TIV [24]. In the present study, children who were seropositive at baseline had significantly higher GMTs after receiving 1 dose of vaccine than did children who were seronegative at baseline after receiving 2 doses of vaccine, suggesting that priming with natural infection may be superior to priming with TIV.

Over one-third of the children in this study had HAI antibody responses <1:40 for the B antigen, even after 2 doses of vaccine. The lower antibody responses to the B vaccine antigen, compared with those to the A vaccine antigens, are consistent with the finding of other studies [16, 28]. Although the lower antibody responses may be due to a reduced immunogenicity of the B antigen component, it is also possible that measuring serologic responses to influenza B virus by HAI may be less sensitive than measuring neutralizing antibody [23, 29]. Gruber et al. administered a single dose of TIV to 6–9-year-old children and demonstrated protection against subsequent, laboratory-confirmed influenza B virus infection. Protection correlated with influenza B neutralizing antibody response [23]. Thus, it is possible that HAI antibody responses to influenza B virus <1:40 may still be protective. Current influenza B viruses fall into 1 of 2 antigenically and genetically distinct lineages, and both B lineages circulated in the 2004–2005 influenza season [30]. The degree of immunity within an influenza virus subtype is dependent on the antigenic relatedness between the variants. As might be expected, TIV induced poor antibody responses to influenza B/Hong Kong, which represents a lineage distinct from that of the vaccine virus strain.

The live attenuated influenza vaccine (LAIV) is licensed in the United States for children 5 years and older. In a community effectiveness study, children 5–9 years of age who received a single dose of LAIV had significantly fewer episodes of medically attended acute respiratory tract illness, compared with LAIV nonrecipients in the intervention community. However, direct comparisons with the data in the present study are limited, because immunogenicity data were not collected as part of the LAIV study, and therefore the proportion of children who had prior exposure to influenza virus through natural infection or prior vaccination was not known [31]. Furthermore, serum antibody response may not be the best or only correlate of protection against influenza after receipt of LAIV [32]. Direct comparisons of LAIV and TIV are needed.

Our data provide additional evidence that influenza vaccines are well tolerated in children [16, 33–35]. Fever in the 5-day period after vaccination was rare, occurring in <1% of children. Injection-site pain, the most commonly reported adverse event, was significantly increased after the second dose of vaccine. In a prior study of 54 children 3–19 years of age receiving a first dose of TIV, 20% reported tenderness at the site of injection that did not limit function, which is much lower than the 59% who reported injection-site pain in this study [23]. The reasons for the more frequent reports of pain in this study are uncertain but may be due to different methods of pain ascertainment, the ages of children, or vaccine antigen components. The proportion of children with pain reported in this study was similar to the proportion of children with pain after other vaccines administered to this age group [36].

These results demonstrate clear immunogenicity benefit of a 2-dose TIV regimen in vaccine-naive 5–8-year-old children when all antigens are considered. Recent vaccine shortages, delays, and prioritization of high-risk groups for early vaccination will challenge the delivery of 2 doses of TIV to healthy 5–8-year-old children and to younger children. At GHC, in the 2001–2002 and 2002–2003 seasons, only 12% (58/495) of previously unvaccinated 5–8-year-old children received 2 doses of influenza vaccine (K. M. Neuzil and L. A. Jackson, unpublished data), and, nationally in 2003–2004, only 8.4% of children 6–23 months old received both necessary doses [37]. Although the majority of children in this study had preexisting antibody to H3N2, the pattern of circulation for H3N2 and other types and subtypes of influenza virus cannot be predicted, and the degree of exposure may vary for children in different regions. Thus, the 2-dose regimen remains the best strategy to prevent influenza illness in young children. More resources should be devoted to improving implementation of the 2-dose regimen for previously unvaccinated children <9 years of age.

Acknowledgments

We are grateful to the children and their parents for participation in the study. We thank Pat Starkovich, Maya Dunstan, Barbara Carste, Thomas Rees, Kamie Beckwith, Amy Wagenseller, Henrietta Hall, and Amanda Balish for their significant contributions to the conduct of the study.

References


